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## Direct Transmission of Human Influenza Virus to Mice.

THOMAS FRANCIS, JR., AND THOMAS P. MAGILL.

*From the Laboratories of the International Health Division, Rockefeller Foundation, New York.*

Repeated attempts have been made to isolate human influenza virus by the direct inoculation of mice with material obtained from patients acutely ill with the disease. Up to the present, however, the establishment of experimental influenza in mice has been successful only when the virus was first subjected to primary passage in ferrets.

In December, 1936, strains of human influenza virus were isolated from fresh cases of influenza occurring in New York. Following one ferret passage, the strains were transferred to mice and became so rapidly adapted to this species of animal that deaths occurred among the mice of the second passage. The ease with which these results were obtained heightened the possibility of transmitting the virus infection directly from human throat washings to mice without intermediate ferret passage.

With plain meat infusion broth as the medium, nasal and pharyngeal washings were collected from 2 patients acutely ill with influenza. The washings were pooled. One portion was used for ferret inoculation and yielded a strain of virus which was readily transferred from ferret to mice. The remainder was stored in the frozen state at —78°C. for 19 days. It was then removed from storage, allowed to thaw at room temperature and centrifugalized at 2500 revolutions per minute for 30 minutes. The supernatant fluid was then centrifugalized in the air-driven centrifuge[1] at 14,000 r.p.m. for 3 hours. The supernatant fluid was removed, the sediment was resuspended in the supernatant to 1/6 the original volume and anesthetized mice were then given 0.05-0.08 cc. of the material intranasally.

Serial passages were made in mice at 4 to 5-day intervals. A heavy suspension (50 to 70%) of the finely ground lung tissue from inoculated mice was given intranasally to normal mice. During the first 3 passages no significant pulmonary lesions were observed. It was shown, however, that virus was present in the lungs of the third passage mice, since a ferret inoculated intranasally with a suspension of these lungs developed the characteristic signs of experimental

[1] Bauer, J. H., and Pickels, E. G., *J. Bact.*, 1936, **31**, 53.

influenza and subsequently developed neutralizing antibodies against human influenza virus. In the 4th passage, the lungs of all the mice presented small areas of involvement. Thereafter, a rapid increase in the virulence of the infectious agent occurred and fatal infection ensued among mice of the 6th passage. For the 7th passage a bacterium-free Berkefeld V filtrate was used. Mice of the 8th passage began to die on the 4th day after their inoculation with a 10% suspension of the involved lung tissue. Neutralization tests, using suspensions of the lungs of mice of the 7th serial passage and known immune serum, clearly identified the infectious agent as human influenza virus. The results are summarized in Table I.

TABLE I.
Direct transfer of human influenza virus from throat washings of influenza patients to mice.

| Passage No. | Date of autopsy | Lung lesions | Remarks |
|---|---|---|---|
| 1 | 1/ 3/37 | 0, +, 0, 0, 0 | |
| 2 | 1/ 7/37 | 0, 0, 0, 0, 0, 0 | |
| 3 | 1/11/37 | 0, 0, 0, 0, 0, 0 | Ferret inoculation positive |
| 4 | 1/16/37 | +++, +, +, +, +, + | |
| 5 | 1/20/37 | +++, ++, ++, ++, ++, ++ | |
| 6 | 1/23/37 | ++++,* ++++, ++++, ++, ++++, +++ | |
| 7 (Filtrate) | 1/28/37 | +++, +++, +++, +++, ++, ++ | Neutralization test done |
| 8 | 2/ 1/37 | ++++,* ++++,* ++++,* ++, ++ | |

Patients: BL 3 and 4. 12/10/36. Original mouse inoculation 12/29/36.
0 to ++++ indicate progressive degrees of lung involvement.
* indicates death of mouse.

It has been possible, therefore, to establish infection with human influenza virus in mice by the direct intranasal inoculation of these animals with throat washings obtained from patients acutely ill with influenza. It is apparent, however, that the procedure as described still lacks certain advantages which accrue to the use of the ferret in which the prompt febrile reaction quickly indicates the presence of active virus. In the case of mice, a comparatively long period exists during which the observer has little or no objective evidence of the presence or absence of virus. Under other conditions, or with other strains, this long period may be shortened or even eliminated.